

Amendments to the Claims

1. (Original) An isolated nucleic acid comprising a sequence encoding a fusion polypeptide, wherein said fusion polypeptide has $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity or $\beta(1,4)$ -galactosyltransferase activity and comprises the Golgi localization domain of a Golgi resident polypeptide.
2. (Original) An isolated nucleic acid according to claim 1, wherein said fusion polypeptide comprises the catalytic domain of $\beta(1,4)$ -N-acetylglucosaminyltransferase III or $\beta(1,4)$ -galactosyltransferase.
3. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of mannosidase II.
4. (Original) An isolated nucleic acid according to claim 3, having the nucleotide sequence shown in Figure 24 and SEQ ID NO:14.
5. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.
6. (Original) An isolated nucleic acid according to claim 5, having the nucleotide sequence shown in Figure 25 and SEQ ID NO:12.
7. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.
8. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of mannosidase I.

9. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.
10. (Original) An isolated nucleic acid according to claim 3 comprising a sequence that encodes a polypeptide having the amino acid sequence shown in Figure 24 and SEQ ID NO: 15.
11. (Original) An isolated nucleic acid according to claim 5 comprising a sequence that encodes a polypeptide having the amino acid sequence shown in Figure 25 and SEQ ID NO:13.
12. (Original) An isolated nucleic acid according to claim 3 comprising a sequence that hybridizes under stringent conditions to a hybridization probe the nucleotide sequence of which consists of the nucleotide sequence shown in Figure 24 and SEQ ID NO:14.
13. (Original) An isolated nucleic acid according to claim 5 comprising a sequence that hybridizes under stringent conditions to a hybridization probe the nucleotide sequence of which consists of the nucleotide sequence shown in Figure 25 and SEQ ID NO:12.
14. (Original) An isolated nucleic acid according to claim 3 comprising a sequence at least 80% identical to the nucleotide sequence shown in Figure 24 and SEQ ID NO:14.
15. (Original) An isolated nucleic acid according to claim 5 comprising a sequence at least 80% identical to the nucleotide sequence shown in Figure 25 and SEQ ID NO:12.
16. (Original) An isolated nucleic acid according to claim 3 comprising a sequence that encodes a polypeptide having an amino acid sequence at least 80% identical to the amino acid sequence of Figure 24 and SEQ ID NO:15.

17. (Original) An isolated nucleic acid according to claim 5 comprising a sequence that encodes a polypeptide having an amino acid sequence at least 80% identical to the amino acid sequence of Figure 25 and SEQ ID NO:13.
18. (Original) An isolated nucleic acid according to claim 3 comprising a sequence that encodes a polypeptide having the amino acid sequence of Figure 24 and SEQ ID NO:15 with conservative amino acid substitutions.
19. (Original) An isolated nucleic acid according to claim 5 comprising a sequence that encodes a polypeptide having the amino acid sequence of Figure 25 and SEQ ID NO:13 with conservative amino acid substitutions.
20. (Currently Amended) An expression vector which comprises an isolated nucleic acid according to ~~any one of claims 1-19~~ claim 1.
21. (Original) A fusion polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity or $\beta(1,4)$ -galactosyltransferase activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide.
22. (Original) A fusion polypeptide according to claim 21, comprising the catalytic domain of $\beta(1,4)$ -N-acetylglucosaminyltransferase III or $\beta(1,4)$ -galactosyltransferase.
23. (Original) A fusion polypeptide according to claim 21, wherein the Golgi localization domain is the localization domain of mannosidase II.
24. (Original) A fusion polypeptide according to claim 21, wherein the Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.
25. (Original) A fusion polypeptide according to claim 21, wherein the Golgi localization domain is the localization domain of mannosidase I.

26. (Original) A fusion polypeptide according to claim 21, wherein the Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.
27. (Original) A fusion polypeptide according to claim 21, wherein the Golgi localization domain is the localization domain of $\alpha 1$ -6 core fucosyltransferase.
28. (Original) A host cell comprising the expression vector of claim 20.
29. (Original) A method for producing a fusion polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity or $\beta(1,4)$ -galactosyltransferase activity comprising culturing the host cell of claim 28 in a medium under conditions allowing the expression of said nucleic acid encoding said fusion polypeptide and recovering said fusion polypeptide from the resultant culture.
30. (Currently Amended) A method for modifying the glycosylation profile of a polypeptide produced by a host cell, comprising introducing into said host cell the nucleic acid of ~~any one of claims 1-19~~ claim 1.
31. (Original) A method for modifying the glycosylation profile of a polypeptide produced by a host cell, comprising introducing into said host cell the expression vector of claim 20.
32. (Original) A method according to claim 30 or 31, wherein said polypeptide is IgG or a fragment thereof.
33. (Original) A method according to claim 32, wherein said polypeptide is IgG1 or a fragment thereof.
34. (Original) A method according to claim 32, wherein said polypeptide is a fusion protein that includes a region equivalent to the Fc region of a human IgG.

35. (Original) A host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity or $\beta(1,4)$ -galactosyltransferase activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide is selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.
36. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell is IgG or a fragment thereof.
37. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell is IgG1 or a fragment thereof.
38. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell is a fusion protein that includes a region equivalent to the Fc region of a human IgG.
39. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell exhibits increased Fc receptor binding affinity as a result of said modification.
40. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell exhibits increased effector function as a result of said modification.
41. (Original) A host cell according to claim 35, wherein said fusion polypeptide comprises the catalytic domain of $\beta(1,4)$ -N-acetylglucosaminyltransferase III or $\beta(1,4)$ -galactosyltransferase.
42. (Original) A host cell according to claim 41, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

43. (Original) A host cell according to claim 42, wherein said Golgi localization domain is the localization domain of mannosidase II.
44. (Original) A host cell according to claim 42, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.
45. (Original) A host cell according to claim 42, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.
46. (Original) A host cell according to claim 42, wherein said Golgi localization domain is the localization domain of mannosidase I.
47. (Original) A host cell according to claim 42, wherein said Golgi localization domain is the localization domain of $\alpha 1$ -6 core fucosyltransferase.
48. (Original) A host cell according to claim 40, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.
49. (Original) A host cell according to claim 40, wherein said increased effector function is increased binding to NK cells.
50. (Original) A host cell according to claim 40, wherein said increased effector function is increased binding to macrophages.
51. (Original) A host cell according to claim 40, wherein said increased effector function is increased binding to polymorphonuclear cells.
52. (Original) A host cell according to claim 40, wherein said increased effector function is increased binding to monocytes.
53. (Original) A host cell according to claim 40, wherein said increased effector function is increased direct signaling inducing apoptosis.

54. (Original) A host cell according to claim 40, wherein said increased effector function is increased dendritic cell maturation.
55. (Original) A host cell according to claim 40, wherein said increased effector function is increased T cell priming.
56. (Original) A host cell according to claim 39, wherein said Fc receptor is Fc γ activating receptor.
57. (Original) A host cell according to claim 39, wherein said Fc receptor is Fc γ RIIIA receptor.
58. (Original) A host cell according to claim 35, wherein said host cell is a CHO cell, a BHK cell, a NSO cell, a SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell or a hybridoma cell.
59. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell is an anti-CD20 antibody.
60. (Original) The host cell of claim 59, wherein said anti-CD20 antibody is IDEC-C2B8.
61. (Original) The host cell of claim 35, wherein said polypeptide produced by said host cell is the chimeric anti-human renal cell carcinoma monoclonal antibody chG250.
62. (Original) The host cell of claim 35, further comprising at least one transected nucleic acid encoding an antibody molecule, and antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.
63. (Original) The host cell of claim 35, wherein said at least one nucleic acid encoding a fusion polypeptide having β (1,4)-N-acetylglucosaminyltransferase III activity

or $\beta(1,4)$ -galactosyltransferase activity is operably linked to a constitutive promoter element.

64. (Original) The host cell of claim 62, wherein said at least one transected nucleic acid encodes an anti-CD20 antibody, the chimeric anti-human neuroblastoma monoclonal antibody chCE7, the chimeric anti-human renal cell carcinoma monoclonal antibody chG250, the chimeric anti-human colon, lung, and breast carcinoma monoclonal antibody ING-1, the humanized anti-human 17-1A antigen monoclonal antibody 3622W94, the humanized anti-human colorectal tumor antibody A33, the anti-human melanoma antibody directed against GD3 ganglioside R24, the chimeric anti-human squamous-cell carcinoma monoclonal antibody SF-25, an anti-human EGFR antibody, an anti-human EGFRvIII antibody, an anti-human PSMA antibody, an anti-human PSCA antibody, an anti-human CD22 antibody, an anti-human CD30 antibody, an anti-human CD33 antibody, an anti-human CD38 antibody, an anti-human CD40 antibody, an anti-human CD45 antibody, an anti-human CD52 antibody, an anti-human CD138 antibody, an anti-human HLA-DR variant antibody, an anti-human EpCAM antibody, an anti-human CEA antibody, an anti-human MUC1 antibody, an anti-human MUC1 core protein antibody, an anti-human aberrantly glycosylated MUC1 antibody, an antibody against human fibronectin variants containing the ED-B domain, or an anti-human HER2/neu antibody.

65. (Original) A method for producing a polypeptide in a host cell, comprising:

- a. culturing a host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having $\beta(1,4)$ -N-acetylglucosaminyltransferase III activity or $\beta(1,4)$ -galactosyltransferase activity under conditions which permit the production of a polypeptide selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin, wherein said fusion polypeptide is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell; and
- b. isolating said polypeptide.

66. (Original) A method according to claim 65 wherein said fusion polypeptide comprises the catalytic domain of $\beta(1,4)$ -N-acetylglucosaminyltransferase III or $\beta(1,4)$ -galactosyltransferase.

67. (Original) A method according to claim 65, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

68. (Original) A method according to claim 67, wherein said Golgi localization domain is the localization domain of mannosidase II.

69. (Original) A method according to claim 67, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

70. (Original) A method according to claim 67, wherein said Golgi localization domain is the localization domain of mannosidase I.

71. (Original) A method according to claim 67, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

72. (Original) A method according to claim 67, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.

73. (Original) A method according to claim 65, wherein said polypeptide has increased effector function as a result of said modification.

74. (Original) A method according to claim 73, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

75. (Original) A method according to claim 73, wherein said increased effector function is increased binding to NK cells.

76. (Original) A method according to claim 73, wherein said increased effector function is increased binding to macrophages.
77. (Original) A method according to claim 73, wherein said increased effector function is increased binding to monocytes.
78. (Original) A method according to claim 73, wherein said increased effector function is increased binding to polymorphonuclear cells.
79. (Original) A method according to claim 73, wherein said increased effector function is direct signaling inducing apoptosis.
80. (Original) A method according to claim 73, wherein said increased effector function is increased dendritic cell maturation.
81. (Original) A method according to claim 73, wherein said increased effector function is increased T cell priming.
82. (Original) A method according to claim 65, wherein said polypeptide produced by said host cell exhibits increased Fc receptor binding affinity as a result of said modification.
83. (Original) A method according to claim 82, wherein said Fc receptor is Fc activating receptor.
84. (Original) A method according to claim 82, wherein said Fc receptor is FcγRIIIA receptor.
85. (Original) A method according to claim 65, wherein said polypeptide produced by said host cell has an increased proportion of bisected oligosaccharides in the Fc region of said polypeptide.

86. (Original) A method according to claim 65, wherein said polypeptide produced by said host cell has an increased proportion of nonfucosylated oligosaccharides in the Fc region of said polypeptide.

87. (Original) A method according to claim 86, wherein said nonfucosylated oligosaccharides are hybrid.

88. (Original) A method according to claim 86, wherein said nonfucosylated oligosaccharides are complex.

89. (Original) A method according to claim 65, wherein said polypeptide produced by said host cell has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide.

90. (Original) A method according to claim 89, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

91. (Original) A method according to claim 89, wherein said bisected, nonfucosylated oligosaccharides are complex.

92. (Original) A method according to claim 89, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

93. (Original) A method according to claim 89, wherein at least 25% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

94. (Original) A method according to claim 89, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

95. (Original) A method according to claim 89, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

96. (Currently Amended) An antibody engineered to have increased effector function produced by the method according to ~~any one of claims 65-96~~ claim 65.
97. (Currently Amended) An antibody engineered to have increased Fc receptor binding affinity produced by the method of ~~any one of claims 65-96~~ claim 65.
98. (Original) An antibody according to claim 97, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.
99. (Original) An antibody according to claim 97, wherein said increased effector function is increased binding to NK cells.
100. (Original) An antibody according to claim 97, wherein said increased effector function is increased binding to macrophages.
101. (Original) An antibody according to claim 97, wherein said increased effector function is increased binding to monocytes.
102. (Original) An antibody according to claim 97, wherein said increased effector function is increased binding to polymorphonuclear cells.
103. (Original) An antibody according to claim 97, wherein said increased effector function is direct signaling inducing apoptosis.
104. (Original) An antibody according to claim 97, wherein said increased effector function is increased dendritic cell maturation.
105. (Original) An antibody according to claim 97, wherein said increased effector function is increased T cell priming.
106. (Original) An antibody according to claim 98, wherein said Fc receptor is Fc activating receptor.

107. (Original) An antibody according to claim 98, wherein said Fc receptor is FcγRIIIa receptor.

108. (Currently Amended) An antibody fragment containing the Fc region and engineered to have increased effector function produced by the method according to ~~any one of claims 65-96~~ claim 65.

109. (Currently Amended) A fusion protein that includes a region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function produced by the method ~~according to any one of claims 65-96~~ of claim 65.

110. (Currently Amended) An antibody fragment containing the Fc region and engineered to have increased Fc receptor binding affinity produced by the method ~~according to any one of claims 65-96~~ of claim 65.

111. (Currently Amended) A fusion protein that includes a region equivalent to the Fc region of an immunoglobulin and engineered to have increased Fc receptor binding affinity produced by the method ~~according to any one of claims 65-96~~ of claim 65.

112. (Currently Amended) A pharmaceutical composition comprising the antibody of ~~any of claims 97-108~~ claim 96 or 97 and a pharmaceutically acceptable carrier.

113. (Original) A pharmaceutical composition comprising the antibody fragment of claim 109 or claim 111 and a pharmaceutically acceptable carrier.

114. (Original) A pharmaceutical composition comprising the fusion protein of claims 110 or 112 and a pharmaceutically acceptable carrier.

115. (Currently Amended) A method for the treatment of cancer comprising administering a therapeutically effective amount of the pharmaceutical composition of any one of claims ~~113-115~~ 112-114 to a patient in need thereof.

116. (Currently Amended) An improved method for disease treatment based on B-cell depletion comprising administering a therapeutically effective amount of antibody to a human subject in need thereof, the improvement comprising administering a therapeutically effective amount of an antibody produced by the method of ~~any one of claims 65-96~~ claim 65.

117. (Currently Amended) The improved method of claim ~~117~~ 116, wherein said antibody is an anti-CD20 monoclonal antibody.

118. (Currently Amended) The improved method of claim ~~118~~ 117, wherein said anti-CD20 antibody is IDEC-C2B8.

119. (Original) An isolated nucleic acid comprising a sequence encoding a fusion polypeptide, wherein said fusion polypeptide has $\beta(1,4)$ -galactosyltransferase activity and comprises the Golgi localization domain of a Golgi resident polypeptide.

120. (Original) An isolated nucleic acid according to claim 119, wherein said fusion polypeptide comprises the catalytic domain of $\beta(1,4)$ -galactosyltransferase.

121. (Original) An isolated nucleic acid according to claim 2, wherein said Golgi localization domain is the localization domain of mannosidase II.

122. (Original) An expression vector which comprises an isolated nucleic acid according to any one of claims 119-121.

123. (Original) A fusion polypeptide having $\beta(1,4)$ -galactosyltransferase activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide.

124. (Original) A fusion polypeptide according to claim 123, comprising the catalytic domain of $\beta(1,4)$ -galactosyltransferase.

125. (Original) A fusion polypeptide according to claim 124, wherein the Golgi localization domain is the localization domain of mannosidase II.

126. (Original) A host cell comprising the expression vector of claim 122.

127. (Original) A method for producing a fusion polypeptide having $\beta(1,4)$ -galactosyltransferase activity comprising culturing the host cell of claim 126 in a medium under conditions allowing the expression of said nucleic acid encoding said fusion polypeptide and recovering said fusion polypeptide from the resultant culture.

128. (Currently Amended) A method for modifying the glycosylation profile of a polypeptide produced by a host cell, comprising introducing into said host cell the nucleic acid of ~~any one of claims 119-121~~ claim 119.

129. (Original) A method for modifying the glycosylation profile of a polypeptide produced by a host cell, comprising introducing into said host cell the expression vector of claim 122.

130. (Original) A host cell comprising:

(a) an expression vector comprising a nucleic acid molecule encoding a fusion polypeptide, wherein said fusion polypeptide has $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnT III) activity and comprises the Golgi localization domain of a Golgi resident polypeptide; and

(b) an expression vector comprising a nucleic acid molecule encoding a polypeptide, wherein said polypeptide has mannosidase II (Man II) activity.

131. (Original) The host cell of claim 130, wherein said nucleic acid molecule encoding said fusion polypeptide and said nucleic acid molecule encoding said polypeptide having mannosidase II activity are on the same expression vector.

132. (Original) The host cell of claim 130, wherein said nucleic acid molecule encoding said fusion polypeptide and said nucleic acid molecule encoding said polypeptide having Man II activity are on separate expression vectors.

133. (Original) The host cell of claim 130, wherein said fusion polypeptide comprises the catalytic domain of GnT III.

134. (Original) The host cell of claim 130, wherein said Golgi localization domain is the localization domain of Man II.

135. (Original) The host cell of claim 130, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

136. (Original) The host cell of claim 130, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

137. (Original) The host cell of claim 130, wherein said Golgi localization domain is the localization domain of mannosidase I.

138. (Original) The host cell of claim 130, wherein said Golgi localization domain is the localization domain of $\alpha 1,6$ -N core fucosyltransferase.

139. (Original) The host cell of claim 130, wherein said host cell is selected from the group consisting of a mammalian cell, a yeast cell, an insect cell or a plant cell.

140. (Original) The host cell of claim 130, wherein said host cell is selected from the group consisting of a CHO cell, a BHK cell, a NSO cell, an SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell, and a hybridoma cell.

141. (Original) The host cell of claim 130, further comprising an expression vector comprising a nucleic acid molecule encoding a polypeptide, wherein said polypeptide has $\beta(1,2)$ -N-acetylglucosaminyl-transferase II (GnT II) activity

142. (Original) The host cell of claim 141, wherein said nucleic acid molecule encoding a fusion polypeptide, said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

143. (Original) The host cell of claim 141, wherein said nucleic acid molecule encoding a fusion polypeptide, said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are each on separate expression vectors.

144. (Original) The host cell of claim 141, wherein said nucleic acid molecule encoding a fusion polypeptide is on one expression vector, and said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

145. (Original) The host cell of claim 141, wherein said nucleic acid molecule encoding a ManII is on one expression vector, and said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

146. (Original) The host cell of claim 141, wherein said nucleic acid molecule encoding said GnT II is on one expression vector, and said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having Man II activity are on the same expression vector.

147. (Original) A host cell comprising:

(a) an expression vector comprising a nucleic acid molecule encoding a fusion polypeptide, wherein said fusion polypeptide has $\beta(1,4)$ -galactosyltransferase (GalT) activity and comprises the Golgi localization domain of a Golgi resident polypeptide; and

(b) an expression vector comprising a nucleic acid molecule encoding a polypeptide, wherein said polypeptide has mannosidase II (Man II) activity.

148. (Original) The host cell of claim 147, wherein said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having mannosidase II activity are on the same expression vector.

149. (Original) The host cell of claim 147, wherein said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having Man II activity are on separate expression vectors.

150. (Original) The host cell of claim 147, wherein said fusion polypeptide comprises the catalytic domain of GalT.

151. (Original) The host cell of claim 147, wherein said Golgi localization domain is the localization domain of Man II.

152. (Original) The host cell of claim 147, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

153. (Original) The host cell of claim 147, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

154. (Original) The host cell of claim 147, wherein said Golgi localization domain is the localization domain of mannosidase I.

155. (Original) The host cell of claim 147, wherein said Golgi localization domain is the localization domain of $\alpha 1,6$ -N core fucosyltransferase.

156. (Original) The host cell of claim 147, wherein said host cell is selected from the group consisting of a mammalian cell, a yeast cell, an insect cell or a plant cell.

157. (Original) The host cell of claim 147, wherein said host cell is selected from the group consisting of a CHO cell, a BHK cell, a NSO cell, an SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell, and a hybridoma cell.

158. (Original) The host cell of claim 147, further comprising an expression vector comprising a nucleic acid molecule encoding a polypeptide, wherein said polypeptide has $\beta(1,2)$ -N-acetylglucosaminyl-transferase II (GnT II) activity

159. (Original) The host cell of claim 158, wherein said nucleic acid molecule encoding a fusion polypeptide, said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

160. (Original) The host cell of claim 158, wherein said nucleic acid molecule encoding a fusion polypeptide, said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are each on separate expression vectors.

161. (Original) The host cell of claim 158, wherein said nucleic acid molecule encoding a fusion polypeptide is on one expression vector, and said nucleic acid molecule encoding a polypeptide having Man II activity and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

162. (Original) The host cell of claim 158, wherein said nucleic acid molecule encoding a ManII is on one expression vector, and said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having GnT II activity are on the same expression vector.

163. (Original) The host cell of claim 158, wherein said nucleic acid molecule encoding said GnT II is on one expression vector, and said nucleic acid molecule encoding a fusion polypeptide and said nucleic acid molecule encoding a polypeptide having Man II activity are on the same expression vector.

164. (Original) The host cell of claim 158, wherein said fusion polypeptide comprises the catalytic domain of GalT.

165. (Original) The host cell of claim 158, wherein said Golgi localization domain is the localization domain of Man II.

166. (Original) The host cell of claim 158, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

167. (Original) The host cell of claim 158, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

168. (Original) The host cell of claim 158, wherein said Golgi localization domain is the localization domain of mannosidase I.

169. (Original) The host cell of claim 158, wherein said Golgi localization domain is the localization domain of $\alpha 1,6$ -core fucosyltransferase.

170. (Original) The host cell of claim 158, wherein said host cell is selected from the group consisting of a mammalian cell, a yeast cell, an insect cell or a plant cell.

171. (Original) The host cell of claim 159, wherein said host cell is selected from the group consisting of a CHO cell, a BHK cell, a NSO cell, an SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell, and a hybridoma cell.

172. (Original) A host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GnT III activity and at least one nucleic acid encoding a polypeptide having Man II activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide produced by said host cell is selected from the group consisting of a

whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

173. (Original) A host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GnT III activity, at least one nucleic acid encoding a polypeptide having Man II and at least one nucleic acid encoding a polypeptide having GnT II activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide produced by said host cell is selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

174. (Original) A host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GalT activity and at least one nucleic acid encoding a polypeptide having Man II activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide produced by said host cell is selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

175. (Original) A host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GalT activity, at least one nucleic acid encoding a polypeptide having Man II and at least one nucleic acid encoding a polypeptide having GnT II activity in an amount sufficient to modify the oligosaccharides in the Fc region of a polypeptide produced by said host cell, wherein said polypeptide produced by said host cell is selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

176. (Original) The host cell according to any of claims 172-175, wherein said polypeptide produced by said host cell exhibits increased Fc receptor binding affinity as a result of said modification.

177. (Original) The host cell of any of claims 172-175, wherein said polypeptide produced by said host cell exhibits increased effector function as a result of said modification.

178. (Original) The host cell according to claim 177, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

179. (Original) The host cell according to claim 177, wherein said increased effector function is increased binding to NK cells.

180. (Original) The host cell according to claim 177, wherein said increased effector function is increased binding to macrophages.

181. (Original) The host cell according to claim 177, wherein said increased effector function is increased binding to polymorphonuclear cells.

182. (Original) The host cell according to claim 177, wherein said increased effector function is increased binding to monocytes.

183. (Original) The host cell according to claim 177, wherein said increased effector function is increased direct signaling induced apoptosis.

184. (Original) The host cell according to claim 177, wherein said increased effector function is increased dendritic cell maturation.

185. (Original) The host cell according to claim 177, wherein said increased effector function is increased T cell priming.

186. (Original) A method for producing a polypeptide in a host cell, comprising:

a. culturing a host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GnT III activity and at least one a nucleic acid encoding a

polypeptide having Man II activity under conditions which permit the production of a polypeptide selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin, wherein said fusion polypeptide is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell; and

b. isolating said polypeptide.

187. (Original) The method of claim 186, wherein said host cell is further engineered to express at least one nucleic acid encoding a polypeptide having GnT II activity.

188. (Original) A method according to claim 186 or 187 wherein said fusion polypeptide comprises the catalytic domain of GnT III.

189. (Original) A method according to claim 188, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

190. (Original) A method according to claim 189, wherein said Golgi localization domain is the localization domain of mannosidase II.

191. (Original) A method according to claim 189, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase I.

192. (Original) A method according to claim 189, wherein said Golgi localization domain is the localization domain of mannosidase I.

193. (Original) A method according to claim 189, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

194. (Original) A method according to claim 189, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.

195. (Original) A method according to claim 186, wherein said polypeptide has increased effector function as a result of said modification.

196. (Original) A method for producing a polypeptide in a host cell, comprising:

- a. culturing a host cell engineered to express at least one nucleic acid encoding a fusion polypeptide having GalT activity and at least one a nucleic acid encoding a polypeptide having Man II activity under conditions which permit the production of a polypeptide selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin, wherein said fusion polypeptide is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell; and
- b. isolating said polypeptide.

197. (Original) The method of claim 197, wherein said host cell is further engineered to express at least one nucleic acid encoding a polypeptide having GnT II activity.

198. (Original) A method according to claim 196 or 197 wherein said fusion polypeptide comprises the catalytic domain of GalT.

199. (Original) A method according to claim 198, wherein said fusion polypeptide further comprises the Golgi localization domain of a heterologous Golgi resident polypeptide.

200. (Original) A method according to claim 199, wherein said Golgi localization domain is the localization domain of mannosidase II.

201. (Original) A method according to claim 199, wherein said Golgi localization domain is the localization domain of β (1,2)-N-acetylglucosaminyltransferase I.

202. (Original) A method according to claim 199, wherein said Golgi localization domain is the localization domain of mannosidase I.

203. (Original) A method according to claim 199, wherein said Golgi localization domain is the localization domain of $\beta(1,2)$ -N-acetylglucosaminyltransferase II.

204. (Original) A method according to claim 199, wherein said Golgi localization domain is the localization domain of α 1-6 core fucosyltransferase.

205. (Original) A method according to claim 199, wherein said polypeptide has increased effector function as a result of said modification.

206. (Original) A method according to claim 186 or 196, wherein said polypeptide produced by said host cell has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide.

207. (Original) A method according to claim 206, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

208. (Original) A method according to claim 206, wherein said bisected, nonfucosylated oligosaccharides are complex.

209. (Original) A method according to claim 206, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

210. (Original) A method according to claim 206, wherein at least 25% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

211. (Original) A method according to claim 206, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

212. (Original) A method according to claim 206, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are bisected, nonfucosylated.

213. (Currently Amended) An antibody engineered to have increased effector function produced by the method of ~~any one of claims 196-212~~ claim 196.

214. (Original) A pharmaceutical composition comprising the antibody of claim 213 and a pharmaceutically acceptable carrier.

215. (Original) A method for the treatment of cancer comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 214 to a patient in need thereof.

216. (Original) A method for producing a polypeptide having increased Fc-mediated cellular cytotoxicity in a host cell, comprising:

a. culturing a host cell engineered to express at least one nucleic acid encoding GalT and at least one nucleic acid encoding Man II under conditions which permit the production of a polypeptide selected from the group consisting of a whole antibody molecule, an antibody fragment that included the Fc region of an immunoglobulin, wherein the expression level of one or both of GalT or Man II is sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell and wherein said polypeptide has increased Fc-mediated cellular cytotoxicity as a result of said modification; and

b. isolating said polypeptide having increased Fc-mediated cellular cytotoxicity.

217. (Original) The method of claim 216, wherein in step (a), said host cell comprises at least one nucleic acid encoding a whole antibody.

218. (Original) The method of claim 216, wherein in step (a), said host cell comprises at least one nucleic acid encoding an antibody fragment.

219. (Original) The method of claim 216, wherein the expression level of GalT produces an antibody molecule or antibody fragment that includes the Fc region of an immunoglobulin having increased Fc-mediated cellular cytotoxicity.

220. (Original) The method of claim 216, wherein said host cell further comprises at least one nucleic acid encoding GnT III, wherein said GnT III is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell and wherein said polypeptide has increased Fc-mediated cellular cytotoxicity as a result of said modification.

221. (Original) The method of any one of claims 216 or 220, wherein the expression level of one or more of GalT, Man II or GnT III is sufficient to form bisected oligosaccharides in the Fc region of said polypeptide.

222. (Original) The method of claim 221, wherein the proportion of bisected oligosaccharides in the Fc region to total oligosaccharides in the Fc region is at least 45 percent.

223. (Original) The method of claim 221, wherein said bisected oligosaccharides are complex.

224. (Original) The method of claim 221, wherein said bisected oligosaccharides are hybrid.

225. (Original) The method of any one of claims 216 or 220, wherein said host cell is selected from the group consisting of a mammalian cell, a yeast cell, an insect cell or a plant cell.

226. (Original) The method of claim 225, wherein said host cell is a plant cell.

227. (Original) The method of any one of claims 216 or 220, wherein said host cell is selected from the group consisting of a CHO cell, a BHK cell, a NSO cell, an SP2/0 cell,

a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell, and a hybridoma cell.

228. (Original) A method for producing a polypeptide in a host cell, comprising:

a. culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having α -Mannosidase II activity under conditions which permit the production of a polypeptide selected from the group consisting of a whole antibody molecule, an antibody fragment, and a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin, wherein said polypeptide having α -Mannosidase II activity is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said polypeptide produced by said host cell; and

b. isolating said polypeptide produced by said host cell.

229. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell has increased effector function as a result of said modification.

230. (Original) A method according to claim 229, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.

231. (Original) A method according to claim 229, wherein said increased effector function is increased binding to NK cells.

232. (Original) A method according to claim 229, wherein said increased effector function is increased binding to macrophages.

233. (Original) A method according to claim 229, wherein said increased effector function is increased binding to polymorphonuclear cells.

234. (Original) A method according to claim 229, wherein said increased effector function is increased binding to monocytes.

235. (Original) A method according to claim 229, wherein said increased effector function is increased direct signaling inducing apoptosis.
236. (Original) A method according to claim 229, wherein said increased effector function is increased dendritic cell maturation.
237. (Original) A method according to claim 229, wherein said increased effector function is increased T cell priming.
238. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell exhibits increased Fc receptor binding as a result of said modification.
239. (Original) A method according to claim 238, wherein said Fc receptor is Fcγ activating receptor.
240. (Original) A method according to claim 238, wherein said Fc receptor is FcγRIIIA receptor.
241. (Original) A method according to claim 228, wherein said host cell is a CHO cell, a BHK cell, a NSO cell, a SP2/0 cell, a YO myeloma cell, a P3X63 mouse myeloma cell, a PER cell, a PER.C6 cell or a hybridoma cell.
242. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell is an anti-CD20 antibody.
243. (Original) A method according to claim 228, wherein said anti-CD20 antibody is IDEC-C2B8.
244. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell is the chimeric anti-human renal cell carcinoma monoclonal antibody chG250.

245. (Original) A method according to claim 228, wherein said host cell further comprises at least one transected nucleic acid encoding an antibody molecule, and antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

246. (Original) A method according to claim 245, wherein said at least one transected nucleic acid encodes an anti-CD20 antibody, the chimeric anti-human neuroblastoma monoclonal antibody chCE7, the chimeric anti-human renal cell carcinoma monoclonal antibody chG250, the chimeric anti-human colon, lung, and breast carcinoma monoclonal antibody ING-1, the humanized anti-human 17-1A antigen monoclonal antibody 3622W94, the humanized anti-human colorectal tumor antibody A33, the anti-human melanoma antibody directed against GD3 ganglioside R24, the chimeric anti-human squamous-cell carcinoma monoclonal antibody SF-25, an anti-human EGFR antibody, an anti-human EGFRvIII antibody, an anti-human PSMA antibody, an anti-human PSCA antibody, an anti-human CD22 antibody, an anti-human CD30 antibody, an anti-human CD33 antibody, an anti-human CD38 antibody, an anti-human CD40 antibody, an anti-human CD45 antibody, an anti-human CD52 antibody, an anti-human CD138 antibody, an anti-human HLA-DR variant antibody, an anti-human EpCAM antibody, an anti-human CEA antibody, an anti-human MUC1 antibody, an anti-human MUC1 core protein antibody, an anti-human aberrantly glycosylated MUC1 antibody, an antibody against human fibronectin variants containing the ED-B domain, an anti-human TAG-72 antibody or an anti-human HER2/neu antibody.

247. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell has an increased proportion of bisected oligosaccharides in the Fc region of said polypeptide.

248. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell has an increased proportion of nonfucosylated oligosaccharides in the Fc region of said polypeptide.

249. (Original) A method according to claim 248, wherein said nonfucosylated oligosaccharides are hybrid.

250. (Original) A method according to claim 248, wherein said nonfucosylated oligosaccharides are complex.

251. (Original) A method according to claim 228, wherein said polypeptide produced by said host cell has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region of said polypeptide.

252. (Original) A method according to claim 251, wherein said bisected, nonfucosylated oligosaccharides are hybrid.

253. (Original) A method according to claim 251, wherein said bisected, nonfucosylated oligosaccharides are complex.

254. (Original) A method according to claim 248, wherein at least 20% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.

255. (Original) A method according to claim 248, wherein at least 25% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.

256. (Original) A method according to claim 248, wherein at least 30% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.

257. (Original) A method according to claim 248, wherein at least 35% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.

258. (Original) A method according to claim 248, wherein at least 40% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.

259. (Original) A method according to claim 248, wherein at least 45% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.
260. (Original) A method according to claim 248, wherein at least 48% of the oligosaccharides in the Fc region of said polypeptide are nonfucosylated.
261. (Original) An antibody engineered to have increased effector function produced by the method of claim 228.
262. (Original) An antibody engineered to have increased Fc receptor binding affinity produced by the method of claim 228.
263. (Original) An antibody according to claim 261, wherein said increased effector function is increased Fc-mediated cellular cytotoxicity.
264. (Original) An antibody according to claim 261, wherein said increased effector function is increased binding to NK cells.
265. (Original) An antibody according to claim 261, wherein said increased effector function is increased binding to macrophages.
266. (Original) An antibody according to claim 261, wherein said increased effector function is increased binding to monocytes.
267. (Original) An antibody according to claim 261, wherein said increased effector function is increased binding to polymorphonuclear cells.
268. (Original) An antibody according to claim 261, wherein said increased effector function is direct signaling inducing apoptosis.
269. (Original) An antibody according to claim 261, wherein said increased effector function is increased dendritic cell maturation.

270. (Original) An antibody according to claim 261, wherein said increased effector function is increased T cell priming.

271. (Original) An antibody according to claim 262, wherein said Fc receptor is Fc activating receptor.

272. (Original) An antibody according to claim 262, wherein said Fc receptor is FcγRIIIa receptor.

273. (Original) An antibody fragment containing the Fc region and engineered to have increased effector function produced by the method of claim 228.

274. (Original) A fusion protein that includes a region equivalent to the Fc region of an immunoglobulin and engineered to have increased effector function produced by the method according to claim 228.

275. (Original) An antibody fragment containing the Fc region and engineered to have increased Fc receptor binding affinity produced by the method according to claim 228.

276. (Original) A fusion protein that includes a region equivalent to the Fc region of an immunoglobulin and engineered to have increased Fc receptor binding affinity produced by the method of claim 228.

277. (Currently Amended) A pharmaceutical composition comprising the antibody of ~~any of claims 261-272~~ claim 261 or 262 and a pharmaceutically acceptable carrier.

278. (Original) A pharmaceutical composition comprising the antibody fragment of claim 273 or claim 275 and a pharmaceutically acceptable carrier.

279. (Original) A pharmaceutical composition comprising the fusion protein of claims 274 or 276 and a pharmaceutically acceptable carrier.

280. (Original) A method for the treatment of a tumor comprising administering a therapeutically effective amount of the pharmaceutical composition of any one of claims 277-279 to a patient in need thereof.

281. (Original) An improved method for disease treatment based on B-cell depletion comprising administering a therapeutically effective amount of antibody to a human subject in need thereof, the improvement comprising administering a therapeutically effective amount of an antibody produced by the method of claim 228.

282. (Original) The improved method of claim 281, wherein said antibody is an anti-CD20 monoclonal antibody.

283. (Original) The method of claim 228, wherein said nucleic acid molecule comprises SEQ ID NO:17.

284. (Original) The method of claim 228, wherein said polypeptide having α -Mannosidase II comprises SEQ ID NO:18.

285. (Original) An isolated nucleic acid molecule according to claim 121 comprising SEQ ID NO:19.

286. (Original) A fusion polypeptide according to claim 125 comprising SEQ ID NO:20.